Appendix E: Scientific Abstract

Mononucleated cells collected from peripheral blood are capable of reconstituting hematopoiesis in hosts whose marrow has been largely ablated, suggesting the presence of hematopoietic repopulating cells in peripheral blood. Whether these cells provide long-term reconstitution, or only short-term repopulation which allows for endogenous reconstitution, has not been firmly established in humans. The purpose of this study is to investigate this question by marking 25% of the peripheral blood repopulating cells (PBRC) before transplantation with the selectable marker gene encoding neomycin phosphotransferase (neo). The neo gene is transduced by cocultivating growth factor mobilized CD34 positive PBRC for 24 hours on lethally irradiated retrovirus-vector producing packaging cells and additional incubation in a vector-containing long-term marrow culture system for 10 days. This transduction protocol has been shown in the dog model to transduce marrow, as well as peripheral blood derived repopulating cells, without any long-term side effects. After conditioning with total body irradiation and/or chemotherapy and subsequent infusion of transduced cells, their contribution to long-term hematopoietic reconstitution will be assessed by analyzing peripheral blood and marrow cells repeatedly for the presence and expression of the marker gene. Cells will be analyzed by PCR for the presence of retrovirus vector DNA. Neo gene expression will be determined by G418-resistant colony assays. These tests will provide information about whether and to what extent the transduced PBRC contribute to long-term hematopoietic reconstitution after transplantation. These studies would help determine the best strategies for transducing retrovirus vector into human hematopoietic repopulating cells, the requisite next step before gene therapy in combination with marrow or PBRC transplantation can be used for treating human disease. Furthermore, the demonstration of long-term contribution of PBRC to hematopoiesis would be of considerable significance because it would enable the use of PBRC alone for autologous and allogeneic transplants and it would suggest the use of PBRC as a long-term carrier of the therapeutically relevant genes in future gene therapy.